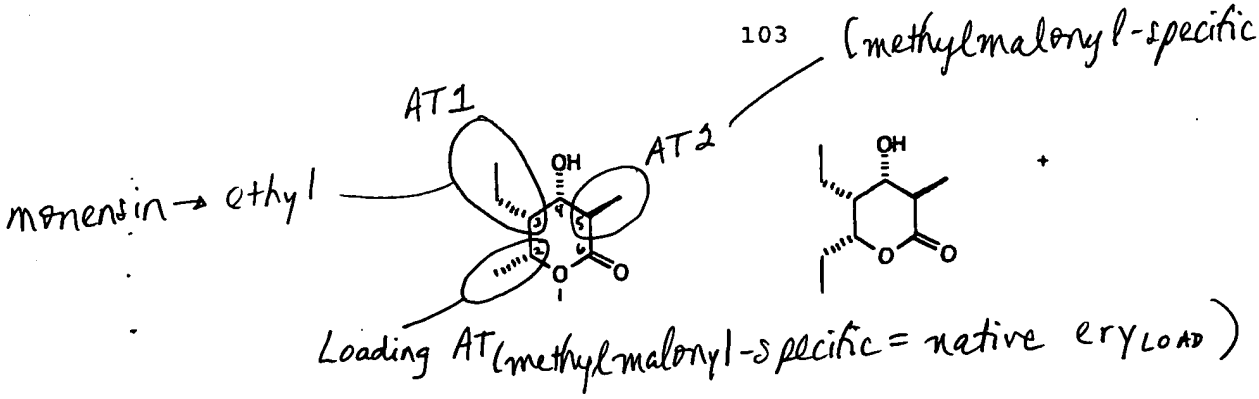

Example 56

Construction of S. erythraea NRRL 2338/pC-ATX and its use in production of 14-membered macrolides

Approximately 5μg pC-ATX DNA was used to transform S. erythraea NRRL2338 protoplasts to give a strain in which the plasmid is integrated into the chromosome. From several colonies, total DNA was obtained and analysed by Southern hybridisation to confirm that the plasmid has integrated in module 2 of EryAI to give a novel macrolide biosynthetic pathway. Further integrations had occurred to give repeated plasmid sequences. S. erythraea NRRL2338 /pC-ATX was inoculated into tryptic soy broth containing 5μg/ml thiostrepton and incubated at 30°C for three days. 100 ml of this seed culture was used to inoculate 2 litres of sucrose succinate defined medium containing 5μg/ ml thiostrepton in 5x 2 litre flasks each containing 500ml medium with 2 springs to aid dispersion and shaken at 300 rpm. After a further 5 days of growth the cultures were centrifuged and the pH of the supernatant adjusted to pH 9. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent removed by evaporation. Products were analysed by HPLC/MS and two macrolide products were identified: